ANNALES HORTICULTURAE
wcześniej – formerly
Annales UMCS sectio EEE Horticultura
VOL. XXVIII (3) 2018

 DOI: 10.24326/ah.2018.3.2

[1] College of Agriculture and Forestry, University of Mosul, Iraq
[2] Department of Plant Protection, University of Life Sciences in Lublin
Leszczyńskiego 7, 20-069 Lublin, Poland
e-mail: aguto@wp.pl, ali_thanoon2005@yahoo.com

ALI HAMOOD THANOON[1, 2], AGNIESZKA JAMIOŁKOWSKA[2]

Effect of mycorrhiza on the diversity and structure of the population of saprotrophic fungi occurring in the rhizosphere of tomato plants

Wpływ mikoryzy na różnorodność i strukturę populacji grzybów saprotroficznych występujących w ryzosferze roślin pomidora

Summary. Arbuscular mycorrhizal fungi (AMF) can be used for protection and stimulation of plant growth. The presence of a symbiont in the roots of plants causes a direct and indirect effect on rhizosphere microorganisms. The aim of studies conducted in 2015–2017 was to estimate the effect of endomycorrhizal fungi such as *Claroideoglomus etunicatum* and *Rhizophagus intraradices* on the population structure of saprotrophic fungi colonizing the rhizosphere of tomato plants cultivated in a plastic tunnel. The experiment was conducted at an ecological farm in Grądy (Lublin district), where the study object were tomato plants ('Antalya F1' – a Turkish hybrid) inoculated by two species of mycorrhizal fungi. The studies showed that AMF had a positive effect on biodiversity of the studied population and on increasing numbers of saprotrophic fungi such as *Trichoderma* spp., *Penicillium* spp., and *Mucor* spp. in the rhizosphere of tomato plant comparing to the control. The mycological analysis of the rhizosphere showed that *C. etunicatum* has a greater impact on the growth of saprotrophic fungi, especially *Trichoderma* spp., in tomato rhizosphere than *R. intraradices*.

Key words: *Claroideoglomus etunicatum, Rhizophagus intraradices*, saprotrophic fungi, mycorrhiza, biocontrol, tomato

INTRODUCTION

Mycorrhiza is known since the 19th century and has been described as obligate biotrophic fungi existed in the plant soil rhizosphere, and as the most common soil microorganisms that can establish mutual symbioses with plants. Root colonization by endomycorrhizal fungi causes changes in the quantity and quality of exudates produced by roots. The mycorrhiza may also affect plants' health status, their competitiveness and succession in ecosystems, and the formation of soil aggregates. The presence of a symbiont in the roots of plants causes a direct and indirect effect on rhizosphere microorganisms, fixing free nitrogen and transforming compounds constituting nutrient substrates for plants [Jamiołkowska *et al.* 2017]. Arbuscular mycorrhizal fungi (AMF) has a significant role in the creation of mycorrhiza. There are the most common form of mutualistic relationships with over 80% of all vascular plants species [Brundrett 2002, Smith and Read 2008, Tahat *et al.* 2010]. Generally, mycorrhizal efficiency is measured in terms of growth status of the host plant [Wu *et al.* 2007]. The most commonly reported benefit of this symbiosis is increased nutrient uptake [Hodge *et al.* 2010]. The AMF network is spreading in the soil and plays an important role in the formation of stable soil aggregates and significantly increases the surface to capture non-viable nutrients [Augé 2001, Brundrett 2004, Johnson *et al.* 2010].

It was shown that the mycorrhizal fungi did not inhibit the activity of the saprotrophic fungi occurring the soil and create possibilities to obtain synergism in the biocontrol of the pathogen through double inoculation – with AMF and the bio-controlling fungus [Joseph and Sivaprasad 2012]. One of the types of saprotrophic fungi are *Trichoderma* species. They inhabit the plant rhizosphere and are biological control organisms against a wide range of soil borne pathogens and provide plant growth promotion. *Trichoderma harzianum* Rifai have been known to show antagonism to various root pathogens such as *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. [Ranasingh *et al.* 2006]. Few studies have focused on the interaction of mycorrhizal fungi with other beneficial antagonist microorganisms like the *Trichoderma* spp. [Vázquez *et al.* 2000].

The aim of the studies was to evaluate the effect of two species of arbuscular mycorrhizal fungi (AMF) – *Claroideoglomus etunicatum* and *Rhizophagus intraradices* – on the population structure of saprotrophic fungi on rhizosphere of tomato plants cultivated in plastic tunnel.

MATERIAL AND METHODS

Agrotechnical experiment. The studies were carried out at the Department of Plant Protection at the University of Life Sciences in Lublin in 2015–2017. A field experiment was conducted at an ecological farm in Grądy (Lublin district) (51°05'36"N 22°12'33"E). The object of studies included tomato plants (*Lycopersicon esculentum* Mill.) 'Antalya F1'. This is a Turkish hybrid cultivar (distribution by Yuksel Tohomculuk Company) for early cultivation under covers. It has strong plants with large leaves and big fruits.

The crop rotation was used in the experiment. The forecrop for each year were cucumber plants. Tomato seedlings were produced at an individual horticulture farm in Lubartów (Lublin district), in accordance with the generally accepted rules for this vegetable. The tomato seedlings were planted in the plastic tunnel in the first decade of May at the spacing of 0.80 × 0.50 m. In each year of studies, the field experiment was established as one-factor experiment in a random block design in 5 replications. Two species of arbuscular mycorrhizal fungi (AMF): *Claroideoglomus etunicatum* W.N. Becker

& Gerd C. Walker & A. Schüssler (CE) and *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler (RI) were used for the inoculation of tomato seedlings. AMF spores were provided by the Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy (Poland). Before planting, seedlings were inoculated with the tested mycorrhizal fungi, introducing spores about 5 cm deep in the rhizosphere of the studied plants. Each inoculum of AMF contained about 25–30 spores per plant. The control combinations were the plants without inoculum of AMF. Black film for mulching has been used in the rows. The drip irrigation was used for irrigation of plants depending on needs. No chemical protection was applied during vegetation period.

Mycological analysis of rhizosphere soil. In 2015–2017 the mycological analysis of the rhizosphere soil of tomato plants were carried out. The soil samples were taken from each experimental combination at the stage of fully ripeness of tomato (first decade of September – BBCH 89) by scratching off the plant roots and placed in sterile Petri dishes. The soil sample from 5 roots for each experimental combination was taken. In the laboratory, the soil samples were mixed into one collective sample, separately for each experimental combination. Next, the rhizosphere soil was shaken in a rotating movement and 1 g of the soil was taken from it and mixed with 74 g of sterilized quartz sand. The mycological analysis of the biodiversity of rhizosphere soil was conducted used Martin medium according to the Warcupa method described by Mańka [1974]. Twenty dishes for each experimental combination were prepared. The dishes were incubated in a thermostat at the temperature of 24°C for 5–7 h in darkness. Fungal colonies grown from the rhizosphere soil were calculated and then transferred to the slants of potato dextrose agar (PDA). Next the colonies of fungi were identified to the species using the available mycological keys and monographs. Fungal nomenclature has used from Index Fungorum database [www.indexfungorum.org].

RESULTS

Biodiversity of fungi colonizing the rhizosphere of tomato plants. During the three-year of mycological analysis of the rhizosphere 1747 colonies of fungi, belonging to 18 species and non-germinating mycelium were isolated (Tab. 1). The communities of fungi colonizing the rhizosphere soil varied. The highest diversity of fungi was observed in the communities in 2015 (21 species) and the lowest in 2017 (13 species) (Tab. 1). *Trichoderma* spp., *Penicillium* spp. and *Fusarium* spp. were the most numerous populations in the rhizosphere of tomato. The most numerous populations of *Fusarium* spp. were recorded in 2017 for control (221 colonies), while the least numerous communities and lack of *Fusarium* spp. were noticed in 2016 for rhizosphere inoculated with AMF (Tab. 1). Mycological analysis of tomato rhizosphere could evaluate the influence of applied AMF on the communities of saprotrophic fungi. As a result of mycological analysis, saprotrophic species such as *Trichoderma* spp., *Penicillium* spp., *Mucor* spp., *Aureobasidium pullulans*, *Humicola fuscoatra*, *Clonostachys rosea* were obtained (Tab. 1). Among the saprotrophic fungi, the most numerous *Trichoderma harzianum* were isolated. AMF had positive effect on the increasing numbers of *Trichoderma* spp. colonies in the rhizosphere of tomato plant inoculated with *C. etunicatum* and *R. intraradices* comparing to the control in each year of experiment (Fig. 1).

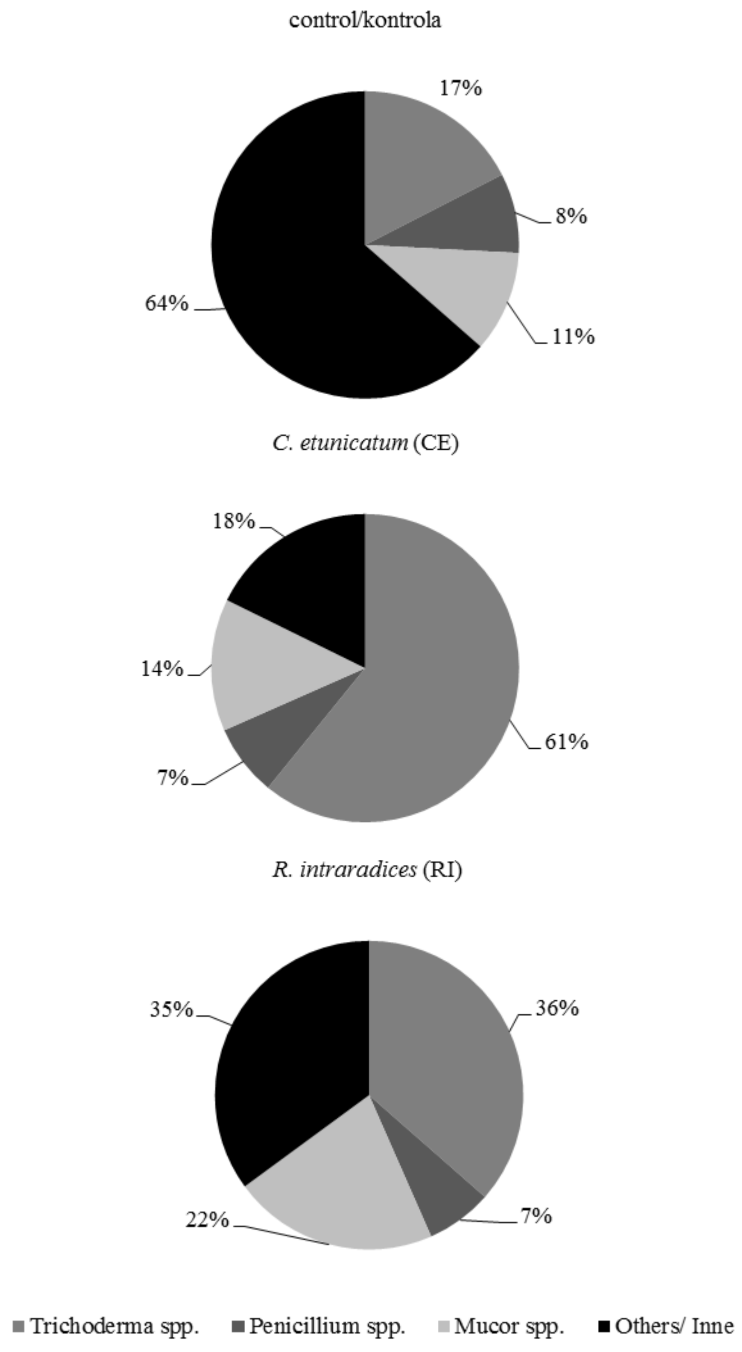

Fig. 1. Percentage of saprophytes in the communities of fungi obtained from the rhizosphere of tomato in 2015–2017
Rys. 1. Procent saprotrofów w zbiorowiskach grzybów uzyskanych z ryzosfery pomidora w latach 2015–2017

Table 1. Number of fungi colonizing rhizosphere of tomato plants ('Antalya F1') in 2015–2017
Tabela 1. Liczba gatunków grzybów zasiedlających ryzosferę roślin pomidora ('Antalya F1') w latach 2015–2017

Year Rok	Fungus species Gatunek grzyba	Experimental combination Kombinacja doświadczalna			Total (%) Razem (%)
		C	CE	RI	
1	2	3	4	5	6
2015	*Acrostalagmus luteoalbus* (Link) Zare. W. Gams & Schroers	6	2	1	9 (2.3)
	Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud	5	–	–	5 (1.0)
	Cephalotrichum microsporum (Sacc.) P.M. Kirk	–	1	–	1 (0.2)
	Chrysosporium merdarium (Ehrenb.) J.W. Carmich.	–	1	3	4 (1.0)
	Cladosporium cladosporioides (Fresen.) G.A. de Vries	8	–	–	8 (2.1)
	Cladosporium herbarum (Pers.) Link	–	–	5	5 (1.0)
	Domingoella asterinarum Petr. & Cif.	4	1	–	5 (1.0)
	Fusarium oxysporum Schltdl.	53	13	23	89 (23.0)
	Humicola fuscoatra Traaen	–	17	3	20 (5.0)
	Mortierella subtilissima Oudem.	1	–	7	8 (2.1)
	Paecilomyces fulvus Stolk & E.S. Salmon	1	–	1	2 (0.5)
	Penicillium albicans Bainier	–	4	–	4 (1.0)
	Penicillium chrysogenum Thom	10	10	–	20 (5.0)
	Penicillium citrinum Thom	–	–	15	15 (3.9)
	Penicillium decumbens Thom	–	–	15	15 (3.9)
	Penicillium simplicissimum (Oudem.) Thom	30	–	–	30 (7.8)
	Thermoascus egyptiacus S. Ueda & Udagawa	4	–	–	4 (1.0)
	Trichoderma hamatum (Bonord.) Bainier	–	57	26	83 (21.7)
	Trichoderma harzianum Rifai	11	21	14	46 (12.0)
	Truncatella spp.	2	–	–	2 (0.5)
	Mycelia sterilia	3	5	–	8 (2.1)
	Total/ Razem	138	132	113	383(100.0)
2016	*Acrostalagmus luteoalbus* (Link) Zare. W. Gams & Schroers	–	4	8	12 (2.3)
	Cladosporium cladosporioides (Fresen.) G.A. de Vries	8	–	–	8 (1.5)
	Clonostachys rosea (Link) Schroers. Samuels. Seifert & W. Gams	1	–	–	1 (0.2)
	Fusarium oxysporum Schltdl.	–	7	–	7 (1.4)
	Fusarium solani (Mart.) Sacc.	2	–	–	2 (0.4)
	Fusarium equiseti (Corda) Sacc.	18	–	–	18 (3.5)

Tab. 1 cont./ cd. tab. 1

1	2	3	4	5	6
	Mucor heterogamus Vuill.	–	13	25	38 (7.4)
	Penicillium aurantiogriseum Dierckx	–	7	–	7 (1.4)
	Penicillium chrysogenum Thom	–	–	10	10 (1.9)
	Penicillium citrinum Thom	–	2	–	2 (0.4)
	Penicillium simplicissimum (Oudem.) Thom	–	12	–	12 (2.3)
	Thermoascus egyptiacus S. Ueda & Udagawa	–	–	4	4 (0.8)
	Trichocladium asperum Harz	10	–	–	10 (1.9)
	Trichoderma aureoviride Rifai	21	2	4	27 (5.2)
	Trichoderma hamatum (Bonord.) Bainier	28	–	–	28 (5.4)
	Trichoderma harzianum Rifai	22	149	145	316 (61.1)
	Verticillium effusum G.H. Otth	–	–	5	5 (1.0)
	Mycelia sterilia	–	10	–	10 (1.9)
	Total/ Razem	110	206	201	517 (100.0
2017	*Acrostalagmus luteoalbus* (Link) Zare. W. Gams & Schroers	3	–	–	3 (0.4)
	Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	14	–	–	14 (1.7)
	Fusarium oxysporum Schltdl.	220	45	18	283 (33.4)
	Fusarium solani (Mart.) Sacc.	–	–	5	5 (0.6)
	Fusarium equiseti (Corda) Sacc.	1	–	60	61 (7.2)
	Mucor circinelloides Tiegh.	38	31	–	69 (8.1)
	Mucor hiemalis Wehmer	23	39	99	161 (19.0)
	Penicillium citrinum Thom	–	10	–	10 (1.2)
	Penicillium expansum Link	8	–	–	8 (0.9)
	Thermoascus egyptiacus S. Ueda & Udagawa	–	–	59	59 (7.0)
	Trichoderma aureoviride Rifai	–	35	–	35 (4.1)
	Trichoderma hamatum (Bonord.) Bainier	–	40	–	40 (4.7)
	Trichoderma harzianum Rifai	18	60	21	99 (11.7)
	Total / Razem	325	260	262	847 (100.0)

C – control/ kontrola, CE – tomato plants inoculated with *Claroideoglomus etunicatum/* rośliny inokulowane *Claroideoglomus etunicatum* RI – tomato plants inoculated with *Rhizophagus intraradices/* rośliny inokulowane *Rhizophagus intraradices*

Population structure of saprotrophic fungi. In the total 674 isolates of *Trichoderma* spp. were obtained during the mycological analysis of soil rhizosphere in 2015–2017, which constitutes from 8.0 to 73.3% of fungi obtained from the studied populations (Tab. 2). The highest number of *Trichoderma* spp. (especially *T. harzianum*, *T. hamatum*, *T. aureoviridae*) were isolated from the rhizosphere of plants inoculated with *C. etunicatum* and *R. intraradices* in 2016 (151 and 149 colonies respectively) compared to control (71 colonies). The lowest number of *Trichoderma* spp. was observed in 2015 and 2017 in the rhizosphere of tomato inoculated with *R. intraradices* (respectively 40 and 21 colonies) compared to control (11 and 18 colonies) (Tab. 2). *Mucor* spp. and *Penicillium* spp. also had a significant participation in the studied fungi communities. *Mucor* spp. (mainly *M. heterogamous*, *M. hiemalis*, *M. circinelloides*) were isolated in 2016–2017 in the number of 268 colonies and were more numerous in the ryzosphere of mycorrhizal plants than in control. *Penicillium* spp. (*P. simplicissimum*, *P. chrysogenum*, *P. citrinum*) were isolated in 2015–2017 in the number of 133 colonies, and they were more numerous in the rhyzosphere of mycorrhized plants than non-mycorrhized ones. The exception was community of fungi in 2015, where the number of *Penicillium* colonies was higher in control than in the soil with mycorrhiza (Tab. 1, 2).

Table 2. Participation of some saprotrophic fungi in the communities obtained from the rhizosphere of tomato in 2015–2017
Tabela 2. Procentowy udział niektórych grzybów saprotroficznych w zbiorowiskach ryzosfery pomidora w latach 2015–2017

Fungi Grzyby	Number of isolates (% of isolates) Liczba izolatów (% izolatów)								
	2015			2016			2017		
	C	CE	RI	C	CE	RI	C	CE	RI
Trichoderma spp.	11 (8.0)	78 (59.1)	40 (35.4)	71 (64.5)	151 (73.3)	149 (74.1)	18 (5.5)	135 (51.9)	21 (8.0)
Penicillium spp.	40 (29.0)	14 (10.6)	30 (26.5)	–	21 (10.2)	10 (5.0)	8 (2.5)	10 (3.8)	–
Mucor spp.	–	–	–	–	13 (6.3)	25 (12.4)	61 (18.8)	70 (26.9)	99 (37.8)
Other	87 (63.0)	40 (30.3)	43 (38.1)	39 (35.5)	21 (10.2)	17 (8.5)	238 (73.2)	45 (17.3)	142 (54.2)
Total	138	132	113	110	206	201	325	260	262

C – control/ kontrola, CE – tomato plants inoculated with *Claroideoglomus etunicatum*/ rośliny inokulowane *Claroideoglomus etunicatum*, RI – tomato plants inoculated with *Rhizophagus intraradices*/ rośliny inokulowane *Rhizophagus intraradices*

The saprotrophic fungi, with antagonistic properties, dominated in the rhizosphere of mycorrhizal plants (CE and RI) of studied communities (2015–2017) and their participation ranged from 65% (RI) to 82% (CE), while in control combination it was only 36% (Fig. 1). The most numerous antagonistic fungi were *Trichoderma* spp. (36 to 61%), while in the control their percentage was only 17% (Fig. 1). Mycological analysis of rhizosphere showed that *C. etunicatum* and *R. intraradices* had a positive effect on the increase of *Trichoderma* spp., *Penicillium* spp. and *Mucor* spp. population in the rhizosphere.

DISCUSSION

Arbuscular mycorrhizal fungi (AMF) create favorable conditions for the activity of rhizosphere microorganisms that have a positive effect on plants. The interactions of microorganisms are visible in soil communities that are highly complex and variable, but very important for plant development. The specific rhizosphere microflora, on the other hand, performs important functions in the activation of spores and mycorrhizal colonization of roots. Mycorrhizal fungi, through the change of the composition and amount of root exudates, have influence on the development and activity of the communities of soil microorganisms [Jamiołkowska *et al.* 2017].

The endomycorrhizal fungi used for the inoculation of tomato plants influenced the increase the biodiversity in the rhizosphere of tomato plants. The number of *Trichoderma* spp., *Penicillium* spp. and *Mucor* spp. colonies in the rhizosphere was greater when tomato plants were inoculated with *C. etunicatum* and *R. intraradices* than in control. Among the saprotrophic fungi, *Trichoderma* genus are important as antagonist. The presence of numerous colonies of *Trichoderma* ssp. in the community have a positive and practical aspect in biological control. Strong competitive abilities of *Trichoderma* spp. resulting from the production of endo- and exoenzymes, toxic metabolites and from overparasitism [Ahmed *et al.* 2009, Jamiołkowska and Thanoon 2016]. Certain soil biocontrolling microorganisms frequently showing synergism of the protective effect on plants together with AMF [Jamiołkowska *et al.* 2017]. *Trichoderma* species, that are common inhabitants of the rhizosphere are biological control organisms against a wide range of soil borne pathogens and also have been known to provide plant growth promotion. *Trichoderma harzianum* have been known to show antagonism to various root pathogens such as *Pythium* spp., *Rhizoctonia* spp. and *Fusarium* spp. [Mwangi *et al.* 2011]. Some strains of *T. harzianum* establish robust and long lasting colonization of root surfaces penetrating into the epidermis [Harman 2000]. Dual inoculation with *T. harzianum* and AMF has previously been reported to significantly enhance growth more than if each fungus was inoculated singly [Šrámek *et al.* 2000, Dubsky *et al.* 2002]. Datnoff and coauthors [1995] show that a combined inoculation of commercial formulations of *G. intraradices* and *T. harzianum* on tomatoes were effective in reducing both the incidence and severity of Fusarium crown and root rot under field conditions.

The rhizosphere region in the combination with applied endomycorrhizal fungi was also colonized by beneficial microorganisms such as *Penicillium* spp. and *Mucor* spp. By means of competition and antibiosis they are able to decrease the severity of pathogens [Nicoletti and Stefano 2012, Lenc *et al.* 2016]. Saprotrophic fungi like the PGPF (plant growth promoting fungi) are beneficial microorganisms, and their synergistic or additive effect could be more valuable than their individual effects. PGPF are non-pathogenic soil fungi effective in controlling several plant diseases [Saldajeno and Hyakumachi 2011].

CONCLUSIONS

The results of the three-years of studies (2015–2017) showed that AMF such as *Claroideoglomus etunicatum* and *Rhizophagus intraradices* had positive effect on the biodiversity of fungal communities and increase the numbers of saprotrophic fungi in the rhizosphere of tomato plants cultivated in plastic tunnels. *Claroideoglomus etunicatum* has a greater impact on the growth of saprotrophic fungi, especially *Trichoderma* spp., in tomato rhizosphere than *R. intraradices*.

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Acknowledgments: The research was carried out with financial support from the University of Mosul in Iraq as part of doctoral studies carried out in 2015–2018

Streszczenie. Arbuskularne grzyby mykoryzowe (AGM) stosuje się w celu ochrony roślin i stymulowania ich wzrostu. Obecność symbionta w korzeniach roślin wpływa bezpośrednio i pośrednio na mikroorganizmy ryzosfery. Celem badań przeprowadzonych w latach 2015–2017 była ocena wpływu grzybów endomykoryzowych, *Claroideoglomus etunicatum* i *Rhizophagus intraradices,* na strukturę populacji grzybów saprotroficznych zasiedlających ryzosferę pomidorów uprawianych w tunelu foliowym. Eksperyment przeprowadzono w gospodarstwie ekologicznym w miejscowości Grądy (województwo lubelskie), w którym przedmiotem badań były rośliny pomidora ('Antalya F1' – hybryda turecka) zaszczepione przez dwa gatunki grzybów mykoryzowych. Badania wykazały, że AGM przyczyniają się do wzrostu bioróżnorodności badanych populacji i powodują zwiększenie liczby grzybów saprotroficznych: *Trichoderma* spp., *Penicillium* spp., *Mucor* spp. w ryzosferze pomidora. Analiza mykologiczna ryzosfery wykazała, że *C. etunicatum* korzystniej wpływa na wzrost liczby grzybów saprotroficznych, w szczególności *Trichoderma* spp., w ryzosferze pomidora niż *R. intraradices*.

Słowa kluczowe: *Claroideoglomus etunicatum, Rhizophagus intraradices*, grzyby saprotroficzne, mykoryza, ochrona biologiczna, pomidor

Received/ Otrzymano: 19.03.2018
Accepted/ Zaakceptowano: 2.09.2018